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(54) Title: SECRETED PROTEINS AND POLYNUCLEOTIDES ENCODING THEM

(57) Abstract

Novel polynucleotides and the proteins encoded thereby are disclosed.

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comprising eight (more preferably twenty, most preferably thirty) contiguous amino acids of SEQ ID NO:70, or a protein comprising a fragment of the amino acid sequence of SEQ ID NO:70 having biological activity, the fragment comprising the amino acid sequence from amino acid 221 to amino acid 230 of SEQ ID NO:70.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:71;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:71 from nucleotide 44 to nucleotide 1513;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:71 from nucleotide 92 to nucleotide 1513;

(d) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:71 from nucleotide 1 to nucleotide 458;

(e) a polynucleotide comprising the nucleotide sequence of the full-length protein coding sequence of clone vp15_1 deposited with the ATCC under accession number 207012;

(f) a polynucleotide encoding the full-length protein encoded by the cDNA insert of clone vp15_1 deposited with the ATCC under accession number 207012;

(g) a polynucleotide comprising the nucleotide sequence of a mature protein coding sequence of clone vp15_1 deposited with the ATCC under accession number 207012;

(h) a polynucleotide encoding a mature protein encoded by the cDNA insert of clone vp15_1 deposited with the ATCC under accession number 207012;

(i) a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:72;

(j) a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:72 having biological activity, the fragment comprising eight contiguous amino acids of SEQ ID NO:72;

(k) a polynucleotide which is an allelic variant of a polynucleotide of (a)-(h) above;

(l) a polynucleotide which encodes a species homologue of the protein of (i) or (j) above ;

(m) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(j); and

(n) a polynucleotide that hybridizes under stringent conditions to any one of the polynucleotides specified in (a)-(j) and that has a length that is at least 25% of the length of SEQ ID NO:71.

Preferably, such polynucleotide comprises the nucleotide sequence of SEQ ID NO:71 from nucleotide 44 to nucleotide 1513; the nucleotide sequence of SEQ ID NO:71 from nucleotide 92 to nucleotide 1513; the nucleotide sequence of SEQ ID NO:71 from nucleotide 1 to nucleotide 458; the nucleotide sequence of the full-length protein coding sequence of clone vp15_1 deposited with the ATCC under accession number 207012; or the nucleotide sequence of a mature protein coding sequence of clone vp15_1 deposited with the ATCC under accession number 207012. In other preferred embodiments, the polynucleotide encodes the full-length or a mature protein encoded by the cDNA insert of clone vp15_1 deposited with the ATCC under accession number 207012. In yet other preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising the amino acid sequence of SEQ ID NO:72 from amino acid 1 to amino acid 139. In further preferred embodiments, the present invention provides a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:72 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) contiguous amino acids of SEQ ID NO:72, or a polynucleotide encoding a protein comprising a fragment of the amino acid sequence of SEQ ID NO:72 having biological activity, the fragment comprising the amino acid sequence from amino acid 240 to amino acid 249 of SEQ ID NO:72.

Other embodiments provide the gene corresponding to the cDNA sequence of SEQ ID NO:71.

Further embodiments of the invention provide isolated polynucleotides produced according to a process selected from the group consisting of:

(a) a process comprising the steps of:

(i) preparing one or more polynucleotide probes that hybridize in 6X SSC at 65 degrees C to a nucleotide sequence selected from the group consisting of:

(aa) SEQ ID NO:71, but excluding the poly(A) tail at the 3' end of SEQ ID NO:71; and

(ab) the nucleotide sequence of the cDNA insert of clone vp15_1 deposited with the ATCC under accession number 207012;

(ii) hybridizing said probe(s) to human genomic DNA in conditions at least as stringent as 4X SSC at 50 degrees C; and

(iii) isolating the DNA polynucleotides detected with the probe(s);

and

(b) a process comprising the steps of:

(i) preparing one or more polynucleotide primers that hybridize in 6X SSC at 65 degrees C to a nucleotide sequence selected from the group consisting of:

(ba) SEQ ID NO:71, but excluding the poly(A) tail at the 3' end of SEQ ID NO:71; and

(bb) the nucleotide sequence of the cDNA insert of clone vp15_1 deposited with the ATCC under accession number 207012;

(ii) hybridizing said primer(s) to human genomic DNA in conditions at least as stringent as 4X SSC at 50 degrees C;

(iii) amplifying human DNA sequences; and

(iv) isolating the polynucleotide products of step (b)(iii).

Preferably the polynucleotide isolated according to the above process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:71, and extending contiguously from a nucleotide sequence corresponding to the 5' end of SEQ ID NO:71 to a nucleotide sequence corresponding to the 3' end of SEQ ID NO:71 , but excluding the poly(A) tail at the 3' end of SEQ ID NO:71. Also preferably the polynucleotide isolated according to the above process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:71 from nucleotide 44 to nucleotide 1513, and extending contiguously from a nucleotide sequence corresponding to the 5' end of said sequence of SEQ ID NO:71 from nucleotide 44 to nucleotide 1513, to a nucleotide sequence corresponding to the 3' end of said sequence of SEQ ID NO:71 from nucleotide 44 to nucleotide 1513. Also preferably the polynucleotide isolated according to the above process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:71 from nucleotide 92 to nucleotide 1513, and extending contiguously from a nucleotide sequence corresponding to the 5' end of said sequence of SEQ ID NO:71 from nucleotide 92 to nucleotide 1513, to a nucleotide sequence corresponding to the 3' end of

said sequence of SEQ ID NO:71 from nucleotide 92 to nucleotide 1513. Also preferably the polynucleotide isolated according to the above process comprises a nucleotide sequence corresponding to the cDNA sequence of SEQ ID NO:71 from nucleotide 1 to nucleotide 458, and extending contiguously from a nucleotide sequence corresponding to the 5' end of said sequence of SEQ ID NO:71 from nucleotide 1 to nucleotide 458, to a nucleotide sequence corresponding to the 3' end of said sequence of SEQ ID NO:71 from nucleotide 1 to nucleotide 458.

In other embodiments, the present invention provides a composition comprising a protein, wherein said protein comprises an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:72;

(b) the amino acid sequence of SEQ ID NO:72 from amino acid 1 to amino acid 139;

(c) a fragment of the amino acid sequence of SEQ ID NO:72, the fragment comprising eight contiguous amino acids of SEQ ID NO:72; and

(d) the amino acid sequence encoded by the cDNA insert of clone vp15_1 deposited with the ATCC under accession number 207012;

the protein being substantially free from other mammalian proteins. Preferably such protein comprises the amino acid sequence of SEQ ID NO:72 or the amino acid sequence of SEQ ID NO:72 from amino acid 1 to amino acid 139. In further preferred embodiments, the present invention provides a protein comprising a fragment of the amino acid sequence of SEQ ID NO:72 having biological activity, the fragment preferably comprising eight (more preferably twenty, most preferably thirty) contiguous amino acids of SEQ ID NO:72, or a protein comprising a fragment of the amino acid sequence of SEQ ID NO:72 having biological activity, the fragment comprising the amino acid sequence from amino acid 240 to amino acid 249 of SEQ ID NO:72.

In one embodiment, the present invention provides a composition comprising an isolated polynucleotide selected from the group consisting of:

(a) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:73;

(b) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:73 from nucleotide 348 to nucleotide 743;

(c) a polynucleotide comprising the nucleotide sequence of SEQ ID NO:73 from nucleotide 414 to nucleotide 743;

Clone "vp15_1"

A polynucleotide of the present invention has been identified as clone "vp15_1". vp15_1 was isolated from a human adult prostate cDNA library and was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. vp15_1 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "vp15_1 protein").

The nucleotide sequence of vp15_1 as presently determined is reported in SEQ ID NO:71, and includes a poly(A) tail. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the vp15_1 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:72. Amino acids 4 to 16 of SEQ ID NO:72 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 17. Due to the hydrophobic nature of the predicted leader/signal sequence, it is likely to act as a transmembrane domain should the predicted leader/signal sequence not be separated from the remainder of the vp15_1 protein. If a "C" residue were inserted between nucleotides 458 and 459 of SEQ ID NO:71, nucleotides 44 to 568 of the resulting nucleotide sequence would encode a protein having an amino acid sequence reported as SEQ ID NO:131.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone vp15_1 should be approximately 2033 bp.

The nucleotide sequence disclosed herein for vp15_1 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. vp15_1 demonstrated at least some similarity with sequences identified as AI033082 (ow97g04.s1 Soares_fetal_liver_spleen_1NFLS_S1 Homo sapiens cDNA clone IMAGE 1654806 3', mRNA sequence) and T21877 (Human gene signature HUMGS03418). The predicted amino acid sequence disclosed herein for vp15_1 was searched against the GenPept and GeneSeq amino acid sequence databases using the BLASTX search protocol. The predicted vp15_1 protein demonstrated at least some similarity to sequences identified as R45335 (Thrombomodulin analogue Q336N, Q365E) and U94333 (C1qR(p) [Homo sapiens]). The predicted vp15_1 protein shows some amino acid similarity to multiple thrombomodulin analogues (such as GeneSeq accession number R45335), and shows some end-to-end similairity to GenPept accession number U94333, which is described as a "... human C1q/MBL/SPA receptor that mediates enhanced phagocytosis *in vitro*" (Nepomuceno *et al.*, 1997, *Immunity* 6(2): 119-129, which

is incorporated by reference herein). Based upon sequence similarity, vp15_1 proteins and each similar protein or peptide may share at least some activity.

vp15_1 protein was expressed in a COS cell expression system, and an expressed protein band of approximately 24 kDa was detected in conditioned medium and membrane fractions using SDS polyacrylamide gel electrophoresis.

Clone "vp17_1"

A polynucleotide of the present invention has been identified as clone "vp17_1". vp17_1 was isolated from a human adult prostate cDNA library and was identified as encoding a secreted or transmembrane protein on the basis of computer analysis of the amino acid sequence of the encoded protein. vp17_1 is a full-length clone, including the entire coding sequence of a secreted protein (also referred to herein as "vp17_1 protein").

The nucleotide sequence of vp17_1 as presently determined is reported in SEQ ID NO:73, and includes a poly(A) tail. What applicants presently believe to be the proper reading frame and the predicted amino acid sequence of the vp17_1 protein corresponding to the foregoing nucleotide sequence is reported in SEQ ID NO:74. Amino acids 10 to 22 of SEQ ID NO:74 are a predicted leader/signal sequence, with the predicted mature amino acid sequence beginning at amino acid 23. Due to the hydrophobic nature of the predicted leader/signal sequence, it is likely to act as a transmembrane domain should the predicted leader/signal sequence not be separated from the remainder of the vp17_1 protein.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone vp17_1 should be approximately 3150 bp.

The nucleotide sequence disclosed herein for vp17_1 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. vp17_1 demonstrated at least some similarity with sequences identified as AI056890 (oz03g07.x1 Soares_fetal_liver_spleen_1NFLS_S1 Homo sapiens cDNA clone IMAGE 1674300 3', mRNA sequence) and T64815 (Tumour suppressor activated pathway gene TSAP6). Based upon sequence similarity, vp17_1 proteins and each similar protein or peptide may share at least some activity. The TopPredII computer program predicts two additional potential transmembrane domains within the vp17_1 protein sequence, one centered around amino acid 50 and another around amino acid 80 of SEQ ID NO:74.

The EcoRI/NotI restriction fragment obtainable from the deposit containing clone vp14_1 should be approximately 1355 bp.

The nucleotide sequence disclosed herein for vp14_1 was searched against the GenBank and GeneSeq nucleotide sequence databases using BLASTN/BLASTX and FASTA search protocols. vp14_1 demonstrated at least some similarity with sequences identified as AI052724 (oz27a12.x1 Soares_total_fetus_Nb2HF8_9w Homo sapiens cDNA clone IMAGE:1676542 3' similar to SW:YQJQ_BACSU P54554 HYPOTHETICAL OXIDOREDUCTASE IN GLNQ-ANSR INTERGENIC REGION; mRNA sequence) and T20001 (Human gene signature HUMGS01138). The predicted amino acid sequence disclosed herein for vp14_1 was searched against the GenPept and GeneSeq amino acid sequence databases using the BLASTX search protocol. The predicted vp14_1 protein demonstrated at least some similarity to sequences identified as R61477 (Clavulanic acid dehydrogenase sequence) and Z99116 (similar to ketoacyl reductase [Bacillus subtilis]). The predicted vp14_1 protein shows some amino acid similarity to various dehydrogenases due to the presence of a short-chain alcohol dehydrogenase family signature at amino acids 51 to 240 of SEQ ID NO:80, as detected by motifs and hidden markov model analysis. Based upon sequence similarity, vp14_1 proteins and each similar protein or peptide may share at least some activity. The TopPredII computer program predicts four additional potential transmembrane domains within the vp14_1 protein sequence, centered around amino acids 55, 195, 230, and 300 of SEQ ID NO:80, respectively.

Deposit of Clones

Clones vb11_1, vb12_1, vb14_1, ve11_1, vf2_1, vg2_1, vj1_1, and vl1_1 were deposited on August 20, 1998 with the ATCC (American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 U.S.A.) as an original deposit under the Budapest Treaty and were given the accession number 98846, from which each clone comprising a particular polynucleotide is obtainable.

Clone vk2_1 was deposited on August 20, 1998 with the ATCC (American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 U.S.A.) as an original deposit under the Budapest Treaty and was given the accession number 98838, from which the vk2_1 clone comprising a particular polynucleotide is obtainable.

Clones vb21_1, vc35_1, vc36_1, vc38_1, vc39_1, vc40_1, vc46_1, vc49_1, vc50_1, vc51_1, and vc52_1 were deposited on September 2, 1998 with the ATCC (American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 U.S.A.) as an original deposit under the Budapest Treaty and were given the accession number 98862, from which each clone comprising a particular polynucleotide is obtainable.

Clones vc33_1, vc34_1, vc47_1, vc54_1, vc57_1, ve13_1, ve16_1, vf3_1, vj2_1, vp7_1, and vp8_1 were deposited on September 22, 1998 with the ATCC (American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 U.S.A.) as an original deposit under the Budapest Treaty and were given the accession number 98886, from which each clone comprising a particular polynucleotide is obtainable.

Clones vb22_1, vc48_1, and vp3_1 were deposited on October 16, 1998 with the ATCC (American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 U.S.A.) as an original deposit under the Budapest Treaty and were given the accession number 98933, from which each clone comprising a particular polynucleotide is obtainable.

Clones vc61_1, vp15_1, vp17_1, vp19_1, and vq1_1 were deposited on December 23, 1998 with the ATCC (American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 U.S.A.) as an original deposit under the Budapest Treaty and were given the accession number 207012, from which each clone comprising a particular polynucleotide is obtainable.

Clone vp14_1 was deposited on December 23, 1998 with the ATCC (American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 U.S.A.) as an original deposit under the Budapest Treaty and was given the accession number 207011, from which the vp14_1 clone comprising a particular polynucleotide is obtainable.

All restrictions on the availability to the public of the deposited material will be irrevocably removed upon the granting of the patent, except for the requirements specified in 37 C.F.R. § 1.808(b), and the term of the deposit will comply with 37 C.F.R. § 1.806.

Each clone has been transfected into separate bacterial cells (*E. coli*) in this composite deposit. Each clone can be removed from the vector in which it was deposited by performing an EcoRI/NotI digestion (5' site, EcoRI; 3' site, NotI) to produce the appropriate fragment for such clone. Each clone was deposited in either the pED6 or pNOTs vector depicted in Figures 1A and 1B, respectively. The pED6dpc2 vector

("pED6") was derived from pED6dpc1 by insertion of a new polylinker to facilitate cDNA cloning (Kaufman *et al.*, 1991, *Nucleic Acids Res.* 19: 4485-4490); the pNOTs vector was derived from pMT2 (Kaufman *et al.*, 1989, *Mol. Cell. Biol.* 9: 946-958) by deletion of the DHFR sequences, insertion of a new polylinker, and insertion of the M13 origin of replication in the ClaI site. In some instances, the deposited clone can become "flipped" (i.e., in the reverse orientation) in the deposited isolate. In such instances, the cDNA insert can still be isolated by digestion with EcoRI and NotI. However, NotI will then produce the 5' site and EcoRI will produce the 3' site for placement of the cDNA in proper orientation for expression in a suitable vector. The cDNA may also be expressed from the vectors in which they were deposited.

Bacterial cells containing a particular clone can be obtained from the composite deposit as follows:

An oligonucleotide probe or probes should be designed to the sequence that is known for that particular clone. This sequence can be derived from the sequences provided herein, or from a combination of those sequences. The sequence of an oligonucleotide probe that was used to isolate or to sequence each full-length clone is identified below, and should be most reliable in isolating the clone of interest.

Clone	Probe Sequence
vb11_1	SEQ ID NO:81
vb12_1	SEQ ID NO:82
vb14_1	SEQ ID NO:83
ve11_1	SEQ ID NO:84
vf2_1	SEQ ID NO:85
vg2_1	SEQ ID NO:86
vj1_1	SEQ ID NO:87
vl1_1	SEQ ID NO:88
vk2_1	SEQ ID NO:89
vb21_1	SEQ ID NO:90
vc35_1	SEQ ID NO:91
vc36_1	SEQ ID NO:92
vc38_1	SEQ ID NO:93
vc39_1	SEQ ID NO:94
vc40_1	SEQ ID NO:95

vc46_1	SEQ ID NO:96
vc49_1	SEQ ID NO:97
vc50_1	SEQ ID NO:98
vc51_1	SEQ ID NO:99
vc52_1	SEQ ID NO:100
vc33_1	SEQ ID NO:101
vc34_1	SEQ ID NO:102
vc47_1	SEQ ID NO:103
vc54_1	SEQ ID NO:104
vc57_1	SEQ ID NO:105
ve13_1	SEQ ID NO:106
ve16_1	SEQ ID NO:107
vf3_1	SEQ ID NO:108
vj2_1	SEQ ID NO:109
vp7_1	SEQ ID NO:110
vp8_1	SEQ ID NO:111
vb22_1	SEQ ID NO:112
vc48_1	SEQ ID NO:113
vp3_1	SEQ ID NO:114
vc61_1	SEQ ID NO:115
vp15_1	SEQ ID NO:116
vp17_1	SEQ ID NO:117
vp19_1	SEQ ID NO:118
vq1_1	SEQ ID NO:119
vp14_1	SEQ ID NO:120

In the sequences listed above which include an N at position 2, that position is occupied in preferred probes/primers by a biotinylated phosphoaramidite residue rather than a nucleotide (such as, for example, that produced by use of biotin phosphoramidite (1-dimethoxytrityloxy-2-(N-biotinyl-4-aminobutyl)-propyl-3-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramadite) (Glen Research, cat. no. 10-1953)).

The design of the oligonucleotide probe should preferably follow these parameters:

(a) It should be designed to an area of the sequence which has the fewest ambiguous bases ("N's"), if any;

(b) It should be designed to have a T_m of approx. 80 ° C (assuming 2° for each A or T and 4 degrees for each G or C).

The oligonucleotide should preferably be labeled with γ-^{32}P ATP (specific activity 6000 Ci/mmole) and T4 polynucleotide kinase using commonly employed techniques for labeling oligonucleotides. Other labeling techniques can also be used. Unincorporated label should preferably be removed by gel filtration chromatography or other established methods. The amount of radioactivity incorporated into the probe should be quantitated by measurement in a scintillation counter. Preferably, specific activity of the resulting probe should be approximately 4e+6 dpm/pmole.

The bacterial culture containing the pool of full-length clones should preferably be thawed and 100 µl of the stock used to inoculate a sterile culture flask containing 25 ml of sterile L-broth containing ampicillin at 100 µg/ml. The culture should preferably be grown to saturation at 37°C, and the saturated culture should preferably be diluted in fresh L-broth. Aliquots of these dilutions should preferably be plated to determine the dilution and volume which will yield approximately 5000 distinct and well-separated colonies on solid bacteriological media containing L-broth containing ampicillin at 100 µg/ml and agar at 1.5% in a 150 mm petri dish when grown overnight at 37°C. Other known methods of obtaining distinct, well-separated colonies can also be employed.

Standard colony hybridization procedures should then be used to transfer the colonies to nitrocellulose filters and lyse, denature and bake them.

The filter is then preferably incubated at 65°C for 1 hour with gentle agitation in 6X SSC (20X stock is 175.3 g NaCl/liter, 88.2 g Na citrate/liter, adjusted to pH 7.0 with NaOH) containing 0.5% SDS, 100 µg/ml of yeast RNA, and 10 mM EDTA (approximately 10 mL per 150 mm filter). Preferably, the probe is then added to the hybridization mix at a concentration greater than or equal to 1e+6 dpm/mL. The filter is then preferably incubated at 65°C with gentle agitation overnight. The filter is then preferably washed in 500 mL of 2X SSC/0.5% SDS at room temperature without agitation, preferably followed by 500 mL of 2X SSC/0.1% SDS at room temperature with gentle shaking for 15 minutes. A third wash with 0.1X SSC/0.5% SDS at 65°C for 30 minutes to 1 hour is optional. The filter is then preferably dried and subjected to autoradiography for sufficient time to visualize the positives on the X-ray film. Other known hybridization methods can also be employed.

The positive colonies are picked, grown in culture, and plasmid DNA isolated using standard procedures. The clones can then be verified by restriction analysis, hybridization analysis, or DNA sequencing.

Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in H.U. Saragovi, *et al.*, Bio/Technology 10, 773-778 (1992) and in R.S. McDowell, *et al.*, J. Amer. Chem. Soc. 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many purposes, including increasing the valency of protein binding sites. For example, fragments of the protein may be fused through "linker" sequences to the Fc portion of an immunoglobulin. For a bivalent form of the protein, such a fusion could be to the Fc portion of an IgG molecule. Other immunoglobulin isotypes may also be used to generate such fusions. For example, a protein - IgM fusion would generate a decavalent form of the protein of the invention.

The present invention also provides both full-length and mature forms of the disclosed proteins. The full-length form of the such proteins is identified in the sequence listing by translation of the nucleotide sequence of each disclosed clone. The mature form(s) of such protein may be obtained by expression of the disclosed full-length polynucleotide (preferably those deposited with the ATCC) in a suitable mammalian cell or other host cell. The sequence(s) of the mature form(s) of the protein may also be determinable from the amino acid sequence of the full-length form.

The present invention also provides genes corresponding to the polynucleotide sequences disclosed herein. "Corresponding genes" are the regions of the genome that are transcribed to produce the mRNAs from which cDNA polynucleotide sequences are derived and may include contiguous regions of the genome necessary for the regulated expression of such genes. Corresponding genes may therefore include but are not limited to coding sequences, 5' and 3' untranslated regions, alternatively spliced exons, introns, promoters, enhancers, and silencer or suppressor elements. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials. An "isolated gene" is a gene that

Additional examples of stringency conditions for polynucleotide hybridization are provided in Sambrook, J., E.F. Fritsch, and T. Maniatis, 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, chapters 9 and 11, and *Current Protocols in Molecular Biology*, 1995, F.M. Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4, incorporated herein by reference.

Preferably, each such hybridizing polynucleotide has a length that is at least 25%(more preferably at least 50%, and most preferably at least 75%) of the length of the polynucleotide of the present invention to which it hybridizes, and has at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with the polynucleotide of the present invention to which it hybridizes, where sequence identity is determined by comparing the sequences of the hybridizing polynucleotides when aligned so as to maximize overlap and identity while minimizing sequence gaps.

The isolated polynucleotide endcoing the protein of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman *et al.*, Nucleic Acids Res. 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, Methods in Enzymology 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed (transfected) with the ligated polynucleotide/expression control sequence.

A number of types of cells may act as suitable host cells for expression of the protein. Mammalian host cells include, for example, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3 cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from in vitro culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells.

Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli, Bacillus subtilis, Salmonella typhimurium*, or any bacterial

strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *e.g.*, Invitrogen, San Diego, California, U.S.A. (the MaxBac® kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (i.e., from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl® or Cibacrom blue 3GA Sepharose®; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the protein of the invention may also be expressed in a form which will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX). Kits for expression and purification of such fusion proteins are commercially available from New England BioLabs (Beverly, MA), Pharmacia (Piscataway, NJ) and Invitrogen Corporation (Carlsbad, CA), respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("Flag") is commercially available from the Eastman Kodak Company (New Haven, CT).

invention will be in the form of a pyrogen-free, parenterally acceptable aqueous solution. The preparation of such parenterally acceptable protein solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art.

The amount of protein of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein of the present invention and observe the patient's response. Larger doses of protein of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01 µg to about 100 mg (preferably about 0.1ng to about 10 mg, more preferably about 0.1 µg to about 1 mg) of protein of the present invention per kg body weight.

The duration of intravenous therapy using the pharmaceutical composition of the present invention will vary, depending on the severity of the disease being treated and the condition and potential idiosyncratic response of each individual patient. It is contemplated that the duration of each application of the protein of the present invention will be in the range of 12 to 24 hours of continuous intravenous administration. Ultimately the attending physician will decide on the appropriate duration of intravenous therapy using the pharmaceutical composition of the present invention.

Protein of the invention may also be used to immunize animals to obtain polyclonal and monoclonal antibodies which specifically react with the protein. As used herein, the term "antibody" includes without limitation a polyclonal antibody, a monoclonal antibody, a chimeric antibody, a single-chain antibody, a CDR-grafted antibody, a humanized antibody, or fragments thereof which bind to the indicated protein.

Such term also includes any other species derived from an antibody or antibody sequence which is capable of binding the indicated protein.

Antibodies to a particular protein can be produced by methods well known to those skilled in the art. For example, monoclonal antibodies can be produced by generation of antibody-producing hybridomas in accordance with known methods (see for example, Goding, 1983, Monoclonal antibodies: principles and practice, Academic Press Inc., New York; and Yokoyama, 1992, "Production of Monoclonal Antibodies" in Current Protocols in Immunology, Unit 2.5, Greene Publishing Assoc. and John Wiley & Sons). Polyclonal sera and antibodies can be produced by inoculation of a mammalian subject with the relevant protein or fragments thereof in accordance with known methods. Fragments of antibodies, receptors, or other reactive peptides can be produced from the corresponding antibodies by cleavage of and collection of the desired fragments in accordance with known methods (see for example, Goding, supra; and Andrew et al., 1992, "Fragmentation of Immunoglobulins" in Current Protocols in Immunology, Unit 2.8, Greene Publishing Assoc. and John Wiley & Sons). Chimeric antibodies and single chain antibodies can also be produced in accordance with known recombinant methods (see for example, 5,169,939, 5,194,594, and 5,576,184). Humanized antibodies can also be made from corresponding murine antibodies in accordance with well known methods (see for example, U.S. Patent Nos. 5,530,101, 5,585,089, and 5,693,762). Additionally, human antibodies may be produced in non-human animals such as mice that have been genetically altered to express human antibody molecules (see for example Fishwild *et al.*, 1996, *Nature Biotechnology* **14**: 845-851; Mendez *et al.*, 1997, *Nature Genetics* **15**: 146-156 (erratum *Nature Genetics* **16**: 410); and U.S. Patents 5,877,397 and 5,625,126). Such antibodies may be obtained using either the entire protein or fragments thereof as an immunogen. The peptide immunogens additionally may contain a cysteine residue at the carboxyl terminus, and are conjugated to a hapten such as keyhole limpet hemocyanin (KLH). Methods for synthesizing such peptides are known in the art, for example, as in R.P. Merrifield, J. Amer.Chem.Soc. 85, 2149-2154 (1963); J.L. Krstenansky, *et al.*, FEBS Lett. 211, 10 (1987).

Monoclonal antibodies binding to the protein of the invention may be useful diagnostic agents for the immunodetection of the protein. Neutralizing monoclonal antibodies binding to the protein may also be useful therapeutics for both conditions associated with the protein and also in the treatment of some forms of cancer where

abnormal expression of the protein is involved. In the case of cancerous cells or leukemic cells, neutralizing monoclonal antibodies against the protein may be useful in detecting and preventing the metastatic spread of the cancerous cells, which may be mediated by the protein.

For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systematically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability.

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